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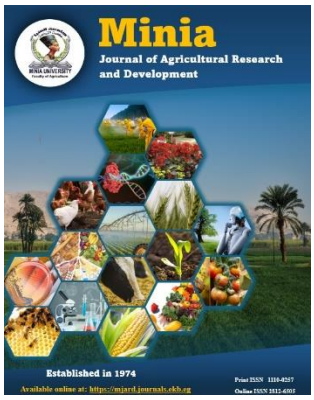

Antioxidant properties and volatile flavor components of flavored fermented soymilk using encapsulated yoghurt starter culture and probiotic bacteria

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ABSTRACT

The antioxidant properties, volatile components and viability of encapsulated bacteria were studied during the storage period for 15 days. Flavored fermented soymilk was prepared from fermented soymilk using encapsulated yogurt starter culture (YSC) and *Bifidobacterium bifidum, 2203 ATCC*, and different mango pulp ratios (0, 10, 20 and 30%). The addition of mango pulp to fermented soymilk using encapsulated bacteria led to an increase in the antioxidant properties, volatile components, and viability of bacteria. The highest increase was noticed in S5 (30% mango pulp + YSC and Bifidobacteria) compared with S1 (0% mango + YSC only) and S2 (0% mango + YSC and Bifidobacteria). Total phenolics content ranged from 66.41 (in S1) to 100.17 (S5) mg GAE/100g, total flavonoids content ranged from 132.67 to 166.56 mg QE/100g, and antioxidant activity ranged from 69.35 to 76.34%. Acetaldehyde content was increased from 2.64 (in S1) to 2.71 PPM (in S2) and decreased with mango pulp at fresh (2.27 PPM in S5) and increased after 15 days of storage period (4.55 PPM in S5). Diacetyl content ranged from 4.33 (S1) to 6.13 PPM (S5). Acetoin content ranged from 8.64 to 9.66 PPM (S5). There was a significant effect of mango pulp addition on the viability of encapsulated bacteria. *Streptococcus thermophilus* ranged from 9.24 to 9.36 (log cfu/ml), *Lactobacillus delbrueckii subsp. bulgaricus* ranged from 9.35 to 9.45 (log cfu/ml), and *Bifidobacterium bifidum 2203 ATCC* ranged from 9.37 to 9.43 (log cfu/ml).

Keywords: Soymilk, mango pulp, encapsulated bacteria, yogurt starter culture and Bifidobacteria.

INTRODUCTION

The consumption of soy food products has increased worldwide as people become more aware of the health benefits of soy-based diets. Soybean products (*e.g.*, soymilk and fermented soymilk) are well established for their rich content of nutritional and phytochemical components, such as isoflavones, phenols, flavonoids, tocopherols, and phytosterols. Consequently, they are known to exhibit potential antioxidant activity **(Rinaldoni *et al.*, 2014; Cai *et al.*, 2021).** Isoflavones have attracted

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a great deal of attention due to their antioxidant, anti-inflammatory, antiallergic, reduce the risk of cardiovascular disease **(Jackman *et al.*, 2007),** promote the inhibition of cancer cell growth, and reduce the menopausal symptoms (because they act as antiestrogens and tyrosine protein kinase inhibitors **(Villares *et al.*, 2011).** Additionally, Soymilk is cholesterol and lactose-free. For these reasons, soymilk is regarded as an ideal substitute to cow's milk by those with milk protein allergies, lactose intolerance, or galactosemia **(Xu & Chang, 2009).** However, despite these benefits, the disagreeable beany flavor, antinutritional factors that reduce mineral absorption, and indigestible oligosaccharides such as stachyose and raffinose, which led to flatulence, limited the consumption of soybean products **(Kumari *et al.*, 2022).** The fermentation of soymilk by LAB improved nutritional value, physicochemical properties, organoleptic qualities and mask the beany flavor of soybean-based products **(Nedele *et al.*, 2021).** Adding mango pulp to fermented soymilk enhances its nutritional value by increasing β-carotene, vitamin C, vitamin B complex, dietary fiber, and folic acid levels. It also provides sweetness and masks the beany flavour of fermented soymilk **(Wale, 2021).** The fermentation of soymilk by lactic acid bacteria (LAB) such as *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* species has improved the total flavonoid content, total phenolic content, antioxidant profile, changed isoflavone profiles, increased aglycone levels from 62 to 96% and exhibited isoflavone distribution and β-glucosidase activity **(Yang *et al.*, 2009).** Over the last two decades, there has been an increasing interest in the role of lactic acid bacteria and probiotic bacteria in human health. To have a positive impact on health, these bacteria must reach their site of action alive and in sufficient numbers to establish themselves (10^6 - 10^7 CFU/g or mL) **(Hill *et al.*, 2014)**. Certain factors affect viability, such as pH, lactic and acetic acid, hydrogen peroxide, and dissolved oxygen level inside the product. For these reasons, the food industry has chosen to develop and implement technologies such as encapsulation to protect microorganisms before and after consumption, thereby ensuring their influence on consumers. Encapsulation of bacteria within polysaccharides such as sodium alginate is an effective technique for use as a bacterial delivery mechanism in food products. It separates the bacterial cell from the adverse environment which leads to reduce cell loss **(Peredo *et al.*, 2016).** In this study, a mixed encapsulated yoghurt starter culture and *Bifidobacterium bifidum* were used to further improve the volatile components, antioxidant properties, and viability of bacteria in fermented soymilk. The microbial composition and the antioxidant properties and volatile flavor substances were quantitatively analyzed after fermentation and during the storage period of fifteen days.

2. MATERIALS AND METHODS

2.1. Materials

1. Raw materials

Soybean seed *(Glycine max L. cv. Giza 111)* were obtained from Agriculture Research Center in Minia governorate, Egypt. Fresh sweet whey produced from the manufacture of Cheddar cheese was obtained from the Department of Dairy Sciences, College of Agricultural, Minia University. Mature, ripe mango *(Mangifera indica cv. Zebdia*) fruits were purchased from a local market in Minya governorate.
The yoghurt starter culture contains *Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus thermophilus*, as well as the probiotic bacteria, *Bifidobacterium bifidum, 2203 ATCC* were obtained from the

American Type Culture Collection (ATCC). Unless otherwise specified, all the reagents and chemicals utilized in the experiment were of analytical grade and purchased from standard commercial sources.

2.Encapsulation of bacteria

The encapsulation method of **Von Mollendorff, (2008) and Frakolaki *et al.*, (2022)** was used with slight modifications. *Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus, and Bifidobacterium bifidum (2203 ATCC)* were cultured in 10 ml MRS and MRSL broth medium at 37 °C for 24 to an optical density (OD 600 nm) of 3.0 (about 1 x 10^9 cfu/ml). The cells were collected by centrifugation at 3000 g for 10 min at 4 °C and rinsed twice with 10 mL of sterile 0.1% (w/v) peptone water. After washing, the cells were suspended in 10 mL of a 2% sterile sodium alginate solution and vortexed until homogeneous. The resulting suspension was transferred to a 20 mL sterile syringe with a 0.45 mm diameter needle and injected dropwise into 100 mL of 0.2 M sterile calcium chloride solution ($CaCl_2$) while gently stirring at 150 rpm. The alginate beads were allowed to stand for 30 min to separate and collected by filtering through Whatman filter paper (No.1), then washed twice with sterile 0.1% (w/v) peptone water and filtered. The beads were stored in sterile 0.1% (w/v) peptone water at 4°C for a maximum of two days.

3. Preparation of Soymilk

Soymilk was prepared according to the procedure described by **Hu *et al.*, (2022),** with some modifications (use sweet whey). One kg of germinated soybeans powder was blended with eight liters of sweet whey (whey/soybeans powder = 8:1, v/w) and ground by electric blender for 10 min. The resulting slurry was filtered through a double-layer muslin cloth to obtain the raw soymilk.

4. Preparation of Mango pulp

Ripe mango fruits *(Mangifera indica cv. Zebdia)* were sorted and washed in tap water, peeled, sliced manually with a sharp knife and then cut into slices. The seeds of mango fruit were removed manually, and the pulp was cut into thin slices. The mango slices were blended by using juice blender without addition of any water, and then filtered by using muslin cloth to separate the fibrous particles to obtain the mango pulp. The mango pulp was pasteurized at 63°C for 30 minutes and cooled immediately and stored at 4°C until preparation of flavored fermented soymilk.

5. Production of flavored fermented soymilk using encapsulated bacteria

flavored fermented soymilk using encapsulated bacteria was prepared according **to Wale, (2021) and D'Alessandro *et al.*, (2023)** with the following modifications. Raw soymilk was heat-treated for 30 minutes at 80-85°C in a regulated boiling water bath; during boiling, sucrose (4% w/v) and pectin (0.2% w/v) were added. The soymilk was constantly stirred during heating. After pasteurization, the soymilk was cooled to the incubation temperature (40-45 ºC) rapidly by a water bath. After cooling, pasteurized soymilk was inoculated with 2% encapsulated yoghurt starter culture (*Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus*) and 2% encapsulated probiotic culture (*Bifidobacterium bifidum, 2203 ATCC*). Inoculated milk was incubated at 42 °C until a pH of 4.6 was reached and this process took approximately 4 h. The previously prepared mango pulp was added into fermented soymilk at 10, 20, and 30% (w/w) concentrations and stirred with a sterile spatula for 5 min. The fermented

soymilk was divided into five treatments as follows:

- **S**1:fermented soymilk using encapsulated yoghurt starter culture.
- **S2:**fermented soymilk using encapsulated (YSC+ Bifidobacteria)
- **S3:**fermented soymilk using encapsulated (YSC+ Bifidobacteria) and 10% mango pulp.
- **S4:**fermented soymilk using encapsulated (YSC+ Bifidobacteria) and 20% mango pulp.
- **S5:**fermented soymilk using encapsulated (YSC+ Bifidobacteria) and 30% mango pulp.

All treatments were put into sterile 100g plastic cups with lids and stored at 4°C for 15 days until sensory analysis.

2.2. Methods

2.2.1. Determination of volatile flavor Components

1. Acetaldehyde Content

Acetaldehyde content of flavored fermented soymilk was measured according to the method described by **Yılmaz, (2006),** To measure the acetaldehyde content, mix the sample to homogenize and weight 10 g into a 250 mL volumetric flask, then add 30 mL of distilled water. The mixture was distilled using a steam distillation apparatus to obtain 10 mL of distillate, after which 1 mL of 0.25 M Sodium bisulfite solution ($NaHSO_3$) was added to bind the acetaldehyde in the distillate, and the pH of the mixture was adjusted to pH 9 with a 0.1 N NaOH solution. The flask was covered and left in a dark place for 15 minutes, 1 mL of 1% starch solution was added as an indicator and titrated with 0.1 N iodine solution until it reached a purple color. Following that, one g sodium bicarbonate ($NaHCO_3$) was added and mixed, and when the mixture became clear, it was titrated again with 0.005 N iodine solution until reached a purple color. The Amount of 0.005 N iodine solution was used to determine the acetaldehyde amount in ppm using the following equation.

$$A\ (ppm) = \frac{44 \times N \times V}{M} \times 100$$

Where:

- A= Amount of acetaldehyde (ppm)
- N= Normality of 0,005 N iodine solution used in titration
- V= Volume of 0,005 N iodine solution used in titration
- M = Weight of sample (gram)

2. Diacetyl and Acetoin content

The diacetyl and acetoin content of flavored fermented soymilk was measured according to the method described by American Society of Brewing Chemists **(ASBC, 1992)**. Weight 20 g of flavored fermented soymilk sample and placed into volumetric flask, and 200 mL of distilled water were added. The mixture was distilled to obtain 5 mL of distillate. A 5 mL of distilled solution was placed into to a test tube, and 1mL of 5 % fresh α-naphthol solution (1g of colorless α-Naphthol powder in 20 ml of 2.5 N NaOH) was added and stirred. After adding 1 mL of 0.5% creatine solution (1g of creatine in 200 ml of distilled water), the mixture was stirred for 1 min. The mixture was left standing for 10 min at room temperature to measure diacetyl and 1h to measure acetoin. The absorbance was assayed using a Spectrophotometer (Turner Model 390 spectrophotometer, Turner, Chicago, USA) at 540 nm against blank. The standard curve of diacetyl and acetoin were used to calculate the concentration of diacetyl and acetoin in samples, and the results were expressed as ppm **(Westerfield, 1945).**

2.2.2. Determination of antioxidant properties:

1. Preparation of Sample extract

The flavored fermented soymilk samples were extracted according to **Guzmán-Ortiz *et al.*, (2017) and Leksono *et al.*, (2022)** with some modification using an acidified methanol solution (methanol: Hydrochloric acid, 99:1.0 v/v). A 2 g of sample was stirred in 10 mL of acidified methanol using a platform shaker at room temperature for 16 hours in total darkness. The mixture was centrifuged at 10,000 g for 10 min at 4 °C (Hettich centrifuge EBA 8, Zentrifugen D-78532 Tuttlingen, Germany). The supernatant was filtered through a small Whatman No. 42 filter paper and stored in the dark at 5 °C for the determination of antioxidant activity, TP content and TF content.

2.Total phenolic content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method according to **Uddin *et al.*, (2015) and El-Galeel *et al.*, (2018)**. Mix 100 µl of sample extract or standard solution at different concentrations with 2.5 ml of 0.2 M Folin-Ciocalteu reagent and leave at ambient temperature for 5 minutes. A solution of sodium bicarbonate 2 ml (75 g/L) was added to the mixture and left at ambient temperature in the dark for 20 min to complete the reaction. The absorbance of the mixture was measured using a UV-visible spectrophotometer (Turner Model 390 spectrophotometer, Turner, Chicago, USA) at 765 nm against blank. Total phenolic content was obtained by measuring the concentration of known standard solution of gallic acid (5 to 100 mg/ml in 80% methanol) by calibration curve. The TPC were calculated and expressed as mg of gallic acid equivalents per gram (mg of GAE/100g).

3. Total Flavonoid Content (TFC)

The total flavonoid content was estimated by the colorimetric method using aluminium chloride reagents **(Bukhari *et al.*, 2008 & Josipovic *et al.*, 2016)**. One milliliter of sample extract was added to a 10 ml volumetric flask containing 4 ml of distilled water, followed by the immediate addition of 0.3 ml of 5% NaNO2. The mixture was vortexed, then left at room temperature for 5 minutes. Following that, 0.3 ml of 10% AlCl3 (w/v) was added, vortexed, and placed at room temperature for 6 minutes. After that, 2 ml of 1 M NaOH and 2.4 ml of distilled water were added and mixed to homogeneity. The absorbance of the pink-colored solution was immediately measured using a UV-visible spectrophotometer (Turner Model 390 spectrophotometer, Turner, Chicago, USA) at 510 nm against blank. Total flavonoids were calculated from a quercetin standard curve and reported as mg quercetin equivalent (QE)/100g of extract.

4. Antioxidant activity

The antioxidant activity was assessed using a DPPH method according to **Al-Saleh *et al.*, (2014) and Leksono *et al.*, (2022)** with slight modifications. To prepare the DPPH solution, 24 mg DPPH was dissolved in 100 mL of methanol and stored as a stock solution at -20°C. The working solution was prepared freshly by mixing 10 milliliters of stock solution with 90 milliliters of methanol. 250 µl of sample extract was mixed with to 4 ml of DPPH working solution. The mixture was shaken vigorously and incubated in the dark at ambient temperature for 30 minutes. The absorbance of the mixture was measured using a UV-visible spectrophotometer (Turner Model 390 spectrophotometer, Turner, Chicago, USA) at 517 nm against a blank containing absolute methanol, and a DPPH working solution was used as a positive control. The DPPH radical-scavenging activity was calculated using the following

equation:

$$\%\text{DPPH scavenging activity} = 1 - \frac{\text{Abs sample}}{\text{Abs control}} \times 100$$

2.2.3 Microbiological analyses

Microbiological analyses of the flavored fermented soymilk were performed to measure the viability of bacteria during storage at 5 °C after 1, 5, 10 and 15 days. Total Viable counts were determined by the standard plate count method using different media and methodologies **(Ismaiel *et al.*, 2018 & Zahrani and Shori,2023).** The media used were MRS agar medium for count of *Lactobacillus delbrueckii ssp. bulgaricus*, M17 agar for count *Streptococcus thermophiles* and MRS agar with 0.05% (w/v) L-cysteine for enumerate *Bifidobacterium bifidum 2203 ATCC*. One milliliter of each sample was aseptically mixed with 9 ml of 0.1% (w/v) sterile peptone water. After that, serial dilutions were prepared up to 10^{-9} using 0.1% sterile peptone water. 1 ml of appropriate dilutions was placed on selective medium using the pour plate method. All plates were incubated at 37 ± 1 ◦C for 48 h and the TVC was expressed as log colony-forming units per milliliter (log cfu/mL) of sample and the viability of bacteria was calculated as follows:

$$\log \text{cfu/ml} = \log\left(\frac{\textit{Number of colonies} \times \textit{dilution factor}}{\textit{volume of sample in plate}(1\text{mL})}\right)$$

$$\%\ \text{Viability} = \frac{\log \text{cfu / ml after 15 days of storage}}{\text{initial log cfu / ml}} \times 100$$

2.2.4. Statistical analysis

The statistical analysis of flavored fermented soymilk treatments and storage period were conducted by Microsoft Excel 365 and the statistical software package of Costate (2005) for Windows (2010) as a two-way ANOVA. Duncan's multiple range test has been used to compare between means and significant differences were accepted at the level of $p \leq 0.05$. The data shown in the tables are the mean values with the standard deviation.

3. RESULTS AND DISCUSSION

1. Volatile flavor Components in flavored fermented soymilk with encapsulated bacteria.

Soymilk fermented only by the yoghurt culture (S1) contained 2.64 ±0.22 ppm of acetaldehyde while in the case of fermented soymilk beverage using yoghurt culture and bifidobacteria (S2) the acetaldehyde content was 2.71 ±0.13 ppm (Table 1). The concentration of acetaldehyde showed insignificant increase ($P > 0.05$) when fermented with yoghurt culture and bifidobacteria (S2) compared to the sample fermented with yoghurt culture only (S1), (Table 1).

This finding is consistent with the results of **Blagden *et al.*, (2005)** who reported that 1.4–3.5 mg/kg acetaldehyde content in fermented soy product using S. thermophilus after 12 h of incubation at 37°C, and almost the results were reported by **Horáčková *et al.*, (2015)** (3.84 ± 0.30 mg/kg). **Horáčková *et al.*, (2015)** reported a low level of acetaldehyde in soymilk fermented by bifidobacteria (only 0.37 ± 0.11 mg/kg). Bifidobacteria strains *(Bifidobacterium animalis subsp. lactis CECT 7953, B. bifidum CCDM 94* and *Bifidobacterium spp. CNRZ 1494)* able to produce acetaldehyde via alcohol dehydrogenase activity, which was capable to oxidize ethanol to acetaldehyde (**Nosova *et al.*, (2000)** and **Margolles and Sanches, (2012).**

As shown in Table (1), When mango pulp was added (S3, S4 and S5), the concentration of acetaldehyde (around 2.35 ppm) decreased when compared with S2 (2.71 ±0.13 ppm). Mango pulp concentration has insignificant effect.

Treatment S5 consistently shows the highest acetaldehyde levels after 10 and 15 days. The concentration of acetaldehyde significantly increased during storage time (15 days) in all treatments. The increase in flavour components with increasing storage period agreed with **Chowdhury, (2020),** who reported that acetaldehyde content gradually increased over storage time and continued during the first six days of storage. Both acetoin (3-hydroxybutan-2-one) and diacetyl (Butane-2,3-dione) confer a yogurt-like odorant and a strong buttery odorant respectively in soymilk fermented products **(Park, & Kim, 2020).** However, the high excess of diacetyl will cause the imbalance of the flavour compounds and unpleasant flavour **(Kaneko et al., 2014 & Wang *et al.*, 2021).**

Results in **Table (1)** showed no significant difference in diacetyl (Butane-2,3-dione) content ($P > 0.05$) between S1 (4.33 ±0.08 ppm) and S2 (4.47 ±0.08 ppm). However, diacetyl content increased significantly with addition of mango pulp into fermented soymilk. Moreover, diacetyl increased significantly during storage period (15 days). The experimental results aligned with the findings of **Peng *et al.*, (2022),** who reported a mean value of 4.35 ± 0.65 for diacetyl (Butane-2,3-dione). **Wang *et al.*, (2021)** reported that diacetyl formation during soymilk fermentation is dependent on the *Lactobacillus* strains. They identified 30 volatile compounds in fermented soymilk. Diacetyl content ranged from 1.39% of total volatile compounds with *Lactobacillus delbrueckii ssp. bulgaricus* to 70.51% with *Lactobacillus plantarum.* Notably, diacetyl was not detected in either non-fermented soymilk or soymilk fermented with *Lactobacillus helveticus*. Their results revealed a decrease in diacetyl content on the first storage day and then remained stable for 7 days at 4°C. Moreover, **Sigüenza-Andrés., *et al* (2024)** reported that formation high levels of diacetyl in fermented products using *Bifidobacterium*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* culture. They attributed the high levels of diacetyl due to the availability of glucose which may favor diacetyl formation.

Acetoin (3-hydroxybutan-2-one) provides powerful creamy and milky flavors to the fermented products. Results in **Table (1)** revealed that there was no significant difference between S1 *(*8.64 ±0.10 ppm) and S2 (8.96 ±0.07 ppm) at day zero, there's a slight difference of 0.32 ppm. The acetoin content increased significantly with addition of mango pulp. S2 sample has a lower acetoin content (8.96 ± 0.07) compared to S3 (9.25 ± 0.13), S4 (9.45 ± 0.12), and S5 (9.66 ± 0.12). This effect was more pronounced with higher mango pulp content (S4 and S5). **Zheng *et al.*, (2020)** reported higher values of acetoin in fermented soymilk using different species from *Lactobacillus, L. harbinensis* M1 produced significantly higher abundance ($P < 0.05$) of acetoin (44.30 ppm). However, **Zong *et al.*, (2022)** found significantly lower levels of acetoin at 11.81 µg/L in a 1.5:1 mixture of fermented soymilk and milk, which subsequently declined to 4.21 and 5.4 µg/L after 15 and 30 days of storage. This may be due to the conversion of acetoin to small amounts of other flavor compounds.

Table 1: Concentration of flavor compounds in flavored fermented soymilk during storage period at 4 ± 1 °C for 15 days.

Storage	Treatments	Acetaldehyde (ppm)	Diacetyl	Acetoin
Zero	S1	2.64 ± 0.22 ^{h}	4.33 ± 0.08 ^{p}	8.64 ± 0.10 ^{k}
	S2	2.71 ± 0.13 ^{h}	4.47 ± 0.08 ^{p}	8.96 ± 0.07 ^{jk}
	S3	2.35 ± 0.13 ^{i}	4.98 ± 0.15 ^{o}	9.25 ± 0.13 ^{ij}
	S4	2.35 ± 0.13 ^{i}	5.77 ± 0.21 ^{m}	9.45 ± 0.12 ^{hij}
	S5	2.27 ± 0.13 ^{i}	6.13 ± 0.19 ^{l}	9.66 ±0.12 ^{ghi}
5days	S1	3.08 ± 0.01 ^{g}	5.32 ± 0.08 ^{n}	9.20 ± 0.18 ^{ijk}
	S2	3.08 ± 0.01 ^{g}	6.41 ± 0.07 ^{k}	10.33 ±1.40 ^{def}
	S3	2.86 ± 0.01 ^{h}	6.98 ± 0.14 ^{j}	9.80 ± 0.11 ^{fghi}
	S4	2.86 ± 0.01 ^{h}	7.58 ± 0.14 ^{i}	10.07 ±0.07 ^{efg}
	S5	2.64 ± 0.01 ^{h}	8.12 ± 0.23 ^{fg}	10.46 ±0.14 ^{de}
10 days	S1	3.63 ± 0.11 ^{f}	7.00 ± 0.17 ^{j}	9.97 ± 0.12 ^{efgh}
	S2	3.74 ± 0.01 ^{f}	7.60 ± 0.17 ^{i}	10.34 ±0.13 ^{def}
	S3	3.81 ± 0.13 ^{ef}	7.93 ± 0.17 ^{gh}	10.88 ±0.16 ^{cd}
	S4	3.96 ± 0.22 ^{de}	8.37 ± 0.25 ^{f}	11.30 ±0.11 ^{bc}
	S5	4.11 ± 0.13 ^{cd}	10.13 ±0.18 ^{c}	11.70 ±0.19 ^{b}
15 days	S1	4.11 ± 0.13 ^{cd}	7.73 ± 0.16 ^{hi}	10.50 ±0.18 ^{de}
	S2	4.18 ± 0.01 ^{c}	8.82 ± 0.21 e	10.91 ±0.12 ^{cd}
	S3	4.25 ± 0.13 ^{bc}	9.44 ± 0.18 ^{d}	11.32 ±0.13 ^{bc}
	S4	4.40 ± 0.22 ^{ab}	10.51 ±0.14 ^{b}	11.87 ±0.12 ^{b}
	S5	4.55 ± 0.13 ^{a}	11.23 ±0.18 ^{a}	12.54 ±0.24 ^{a}

Values with the same letter in each column are not-significant differences.

2. Antioxidant properties of flavored fermented soymilk with encapsulated bacteria.

Results in **Table (2)** show the changes in total phenolic, total flavonoids and antioxidant activity of flavored fermented soymilk. Total phenolic compounds significantly increased ($P < 0.05$) in S2 sample **(74.27 ± 2.55 mg GAE/100g)** compared with S1**(66.41 ± 3.84 mg GAE/100g)** at fresh. It seems that the addition of Bifidobacteria (S2) significantly increases total phenolic content compared to yoghurt culture alone (S1) at fresh. The addition of mango pulp increased the total phenolic compound, and this increment was proportionally with the percentage of added mango.

Maldonado-Celis *et al.*, (2019) determined the phenolic compounds in fresh mango (FW) pulp and found that the highest phenolic acid in 100 g FW of pulp was ferulic acid (33.75 mg), followed by chlorogenic (0.96–6.20 mg), gallic (0.93–2.98 mg), vanillic (0.57–1.63 mg), protocatechuic (0.77 mg), and caffeic acids (0.25–0.10 mg) Moreover, the results revealed a gradual increase in phenolic compounds during storage period.

The highest increases for phenolic compounds were observed in the samples fermented with high mango pulp (S5),

(Table 2). The obtained values are in close with those obtained by **Csatlos *et al.*, (2023)** where the concentration of polyphenols in fermented soybean beverage was 306.72 µg GAE/ mL. It is essential to mention that in this work, the beverages that had been fermented with encapsulated bacteria had a high viability of LAB, which could be responsible for that, along with the phenolic released from culture bacteria during storage. Additionally, **de Queirós *et al.*, (2020)** found that the phenolic content of unfermented soymilk control was 1132 µg GAE/mL which increased to 1442 µg GAE/mL with *Lb. bulgaricus* MB153 and 1408 µg GAE/mL with *Str. thermophilus* ST 066.

Concerning flavonoids content, addition of bifidobacteria increased flavonoids values from 132.67±6.67 in S1 to 137.67±3.34 mg quercetin equivalent/100g in S2. Moreover, the levels of flavonoids significantly increased with addition of mango pulp, with the highest level in S5 (30% mango pulp) 166.56 ±4.19 mg quercetin equivalent/100g on fresh. Storage time leads to a decrease in total flavonoids for all samples, with a more pronounced effect in samples without mango pulp. Flavonoid contents decreased by about 40.21, 35.12, 29.61, 22.16 and 20.35% in S1, S2. S3. S4 and S5 respectively after 15-day storage **(Table 3).** The rate of decrease was low with mango addition. **Kašparovská *et al.*, (2017)** found that the daidzein concentration in the fresh yoghurt samples decreased after fermentation by about 36-53%. Moreover, **Pyo and Song, (2009)** reported that the total isoflavone content in yoghurt decreased by approximately 14.3% after 30 days of storage. However, **Pham and Shah, (2009),** found that 4 isoflavone glucosides and 3 isoflavone aglycones studied in their work were considerably resistant over 28 d of storage at 4 °C.

Peng *et al.*, (2022) investigated the dynamic changes in the three primary isoflavones daidzein, genistein glycosides, and aglycones within soymilk fermented by lactic acid bacteria over a 48-days storage period. Their findings indicated that daidzein and genistein levels increased post-fermentation but subsequently declined during storage. Conversely, aglycone concentrations exhibited an increment due to fermentation and continued to gradually rise throughout the storage period. This phenomenon is attributed to the enzymatic activity of β-glucosidase, released by lactic acid bacteria, which catalyzes the conversion of isoflavone glycosides into their corresponding aglycone forms. In a separate study, **de Queirós *et al.*, (2020)** observed a substantial increase in isoflavone content following 24 hours of fermentation. Specifically, daidzein, genistein, and glycitein levels amplified by 10.3- to 13.1-fold, 10.4- to 12.3-fold, and 3.8- to 4.7-fold, respectively, when compared to the control soymilk.

Antioxidant Activity of flavored fermented soymilk presented in **Table 2** showed that the antioxidant activity of S2 (LAB + bifidobacteria) shows a higher antioxidant activity (71.27 ± 0.79) compared to S1 (LAB) with a value of 69.35 ± 0.70. That means that addition of bifidobacteria to the fermented soymilk beverage appears to enhance its antioxidant capacity. This suggests that bifidobacteria may contribute to the production of antioxidant compounds or promote the preservation of existing antioxidants during the fermentation process. **Shen Qian *et al.*, (2011)** reported that the *Bifidobacterium animalis* 01 significantly enhanced the antioxidative enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) and reduce Malondialdehyde (MDA) and exhibited stronger scavenging

effect. Moreover, **Li *et al.*, (2019)** stated that the milk fermented with *Bif. animalis* ssp. *Lactis* had higher antioxidant activity compared to milk fermented with regular starter cultures. **Peng *et al.*, (2022)** reported higher antioxidant activity (75.49 %), in fermented soybean using mixed starter culture (LAB + bifidobacteria) compared to 69.07 % for those fermented with LAB alone. These findings are close to our results in **Table (2)** (71.27 ±0.79%). Fermented soymilk exhibits potent reducing properties, enabling it to neutralize free radicals by donating electrons. This process effectively interrupts the chain reaction of free radical propagation, thereby demonstrating its antioxidant capabilities **(Peng *et al.*, 2022).** Furthermore, **Zhang et al. (2018)** found that microbial fermentation enhances the production of proteases, including endopeptidases and peptidases. These enzymes break down proteins into smaller peptides, which significantly contribute to the antioxidant properties of the final product.

Table 2- Total phenolic, Total flavonoids and Antioxidant activity in flavored fermented soymilk during storage period at 4 ± 1 °C for 15 days.

Storage	Treatments	Total phenolics (mg GAE/100g)	Total flavonoids (mg QE/100g)	Antioxidant activity (%)
Zero	S1	66.41 ±3.84 ^{l}	132.67 ±6.67 ^{e}	69.35 ±0.70 ^{k}
	S2	74.27 ±2.55 ^{k}	137.67 ±3.34 ^{de}	71.27 ±0.79 ^{j}
	S3	77.03 ±3.01 ^{jk}	148.22 ±3.47 ^{bc}	72.90 ±0.70 ^{i}
	S4	92.10 ±2.55 ^{fg}	155.45 ±2.55 ^{b}	74.24 ±0.27 ^{gh}
	S5	100.17 ±3.90 ^{de}	166.56 ±4.19 ^{a}	76.34 ±0.44 ^{de}
5days	S1	75.76 ±3.01 ^{jk}	102.67 ±3.34 ^{g}	71.15 ±0.80 ^{j}
	S2	82.97 ±2.24 ^{hi}	103.22 ±5.85 ^{g}	72.90 ±0.70 ^{i}
	S3	87.43 ±4.52 ^{gh}	132.67 ±3.34 ^{e}	74.71 ±0.53 ^{fg}
	S4	98.68 ±3.27 ^{e}	143.22 ±5.85 ^{cd}	75.35 ±0.35 ^{ef}
	S5	108.24 ±3.21 ^{c}	154.33 ±3.34 ^{b}	77.62 ±0.35 ^{c}
10 days	S1	81.06 ±1.94 ^{ij}	89.89 ±4.19 ^{h}	73.37 ±0.79 ^{hi}
	S2	89.34 ±2.23 ^{g}	99.89 ±4.19 ^{g}	74.59 ±0.61 ^{fg}
	S3	95.92 ±2.55 ^{ef}	114.89 ±6.73 ^{f}	76.22 ±0.46 ^{de}
	S4	107.39 ±3.37 ^{c}	131.56 ±5.09 ^{e}	76.81 ±0.36 ^{cd}
	S5	114.82 ±1.95 ^{b}	141.56 ±5.85 ^{cd}	78.85 ±0.35 ^{b}
15 days	S1	88.92 ±2.30 ^{g}	79.33 ±3.34 ^{i}	75.35 ±0.52 ^{ef}
	S2	97.41 ±5.11 ^{ef}	89.33 ±3.34 ^{h}	76.40 ±0.53 ^{d}
	S3	105.05 ±2.58 ^{cd}	104.33 ±5.00 ^{g}	77.57 ±0.53 ^{c}
	S4	114.18 ±2.65 ^{b}	121.00 ±5.00 ^{f}	78.79 ±0.53 ^{b}
	S5	125.22 ±3.82 ^{a}	132.67 ±3.34 ^{e}	80.42 ±0.52 ^{a}

Values with the same letter in each column are not-significant differences.

With addition of mango pulp the antioxidant activity of fermented soymilk increases as the percentage of mango pulp increases from S3 to S5, there is a consistent increase in antioxidant activity. S3 (10% mango pulp) has a higher value than S2, and this trend continues with S4 (20% mango pulp) and S5 (30% mango pulp) showing progressively higher antioxidant activity. This could be attributed to the presence of antioxidants naturally found in mango pulp, such as polyphenols and carotenoids. Increasing the mango pulp content leads to a greater concentration of these antioxidants, resulting in higher overall antioxidant activity.

Santhirasegaram *et al.*, (2015) found that mango pulp has high levels of phenolic compounds like caffeoyl glucose, quinic acid/chinic acid, monogalloyl glucose, ellagic acid, quercetin, gallic acid, kaempferol, mangiferin, tannic acid/gallotannin with 1022 µg of ascorbic acid equivalent (AAE/ml) antioxidant activity. Gallotannin (tannic acid) is a hydrolysable tannin, and easily converted into gallic acid when oxidized. Gallic acid is well known for its high antioxidant capacity. They added these compounds are highly stable during storage for 5 weeks at 4 ± 1 °C.

Table 3- Decrease rate of total flavonoids in flavored fermented soymilk after 15 days at 4 ± 1 °C.

% Decrease of total flavonoids after 15 days storage					
Treatments	S1	S2	S3	S4	S5
% Decrease after 15 days	40.21	35.11	29.61	22.16	20.35

3: Viability of encapsulated bacteria in flavored fermented soymilk.

The efficacy of probiotic products is directly linked to the survival of live microorganisms from production to consumption. This critical parameter is quantified as Minimum Bio-value (MBV), representing the minimum viable cell count necessary for a product's beneficial effects (10^6 - 10^7 cfu/ml) which increased to 1×10^9 cfu/g by Health Canada **(Hill *et al.*, 2014)**. However, maintaining high probiotic viability is fraught with challenges. Numerous factors influence probiotic survival, including production conditions (pH, temperature, nutrients (**Cano-Lozano *et al.*, 2022**), storage (temperature, light), product formulation, and the harsh gastrointestinal environment (**Mendon *et al.*, 2022 and Gökırmaklı *et al.*, 2024).** Fermented milks, in particular, present significant hurdles due to slow bacterial growth and detrimental conditions during production and storage. Consequently, probiotic counts often dwindle below recommended levels at consumption, compromising their potential benefits. Encapsulation is therefore, the most important factor to keep viability of bacteria higher than the Minimum Bio-value (MBV), The results in tables (4, 5 and 6) represent the total viable counts of *Lactobacillus delbrueckii* spp. *bulgaricus*, *Streptococcus thermophilus* and *Bifidobacterium bifidum*

(2203 ATCC) in different fermented soymilk samples over a storage period of 15 days.

The results in **table (4)** represent the total bacterial count (log cfu/ml) of *Lactobacillus delbrueckii* spp *bulgaricus* in different fermented soymilk samples containing mango pulp (S1 to S5) over a 15-days storage period. No significant difference between viability of *Lactobacillus* in S1 and S2 but adding bifidobacteria (S2) seems to slightly increase *Lactobacillus* counts compared to the yogurt culture alone (S1). Moreover, the addition of mango pulp (S3, S4, S5) does not significantly affect.

Lactobacillus growth compared to S2. The total bacterial count increased during storage, from 9.35 ±0.03, 9.38 ±0.02, 9.39 ±0.02, 9.41 ±0.03 and 9.45 ±0.01 log cfu/ml in S1, S2, S3, S4 and S5 respectively, in fresh samples to 13.43 ±0.02, 13.45 ±0.02, 13.45 ±0.01, 13.47 ±0.01 and 13.48 ±0.01 log cfu/ml in the same order after 15 days of storage. The same trend was observed for *Streptococcus thermophilus* and *Bifidobacteria* **(table 5 and 6).** This is counterintuitive to what is typically expected, as bacterial populations often stabilize or decline over time due to factors like nutrient depletion and waste accumulation. The increased viability of *Lactobacillus* bacteria during storage can be attributed to the encapsulation of bacteria used in these experiments.

Encapsulating bacteria significantly enhances their viability during storage by protecting them from environmental stressors such as oxygen, light, heat, and acidic conditions. The encapsulation matrix can be designed to release bacteria gradually, maintaining a sustained population over time. Moreover, encapsulation creates a microenvironment within the capsule that allows bacteria to sustain higher metabolic activity and better tolerate storage conditions **Lopes *et al.*, (2020)** found that FTIR analysis revealed an interaction between alginate and the cell wall of the immobilized microorganism, enhancing its stability. Furthermore, **Pereira *et al.*, (2011)** found that the viability of *Lb. casei NRRL B-442* was higher than 8.00 log CFU/mL after storage for 42 days at 4°C, the addition of fruit juice (apple juice) improved the growth of *Lb. mesenteroides* and *Lb. johnsonii* **(Vergara *et al.*,2010).**

Rai and Bai, (2015) reported that the encapsulated *Lactobacillus delbrueckii* subsp. *bulgaricus* maintained maximum cell counts (2.9-3.9 × 10^8 CFU/mL) for two weeks of storage at 4°C, but populations declined after four weeks. The encapsulation in this type of microsphere significantly increased the viability of the microorganisms even at low pH (3.80). The diameter of the capsules must be sufficiently large (0.2–3 mm to protect the bacterial content but also small enough to prevent adverse alterations in the sensory profile of the food **(Rai and Bai, 2015 and Mendon *et al.*, 2022).** The results of the present study are in accordance with **Afzaal et al., (2020)** who studied the storage stability of probiotic bacteria and reported that encapsulation process is an effective approach to enhance the survival of probiotics under stressed conditions.

D'Alessandro *et al.*, (2023) found that the viability of encapsulated *Streptococcus thermophilus* increased in fermented soybean beverage stored at 4°C for 14 days especially when added with probiotic bacteria as adjunct culture. It seems that the encapsulation matrix around living cells of microorganisms provides them with a physical barrier and hydration capacity against harsh environmental conditions **(Burgain *et al.*, 2011). Naklong *et al.*, (2023)** demonstrated that encapsulating probiotic bacteria in sodium alginate

microcapsules improved bacterial cell viability in the yoghurt during 20 days of storage at 4 ◦C. The denser surface morphology of alginate-dairy microcapsules likely contributes to their ability to protect encapsulated cells from harmful external conditions. The efficacy of encapsulated probiotics in withstanding the harsh conditions of simulated gastric fluid was positively correlated with bead size.

Table 4: Total viable counts of *Lactobacillus delbrueckii* spp. *bulgaricus,* of flavored fermented soymilk during storage period at 4 ± 1 °C for 15 days (log cfu/g).

Storage period (day)	*Lactobacillus delbrueckii* spp *bulgaricus* (log cfu/ml)				
	S1	S2	S3	S4	S5
Fresh	9.35 ±0.03 ^{l}	9.38 ±0.02 ^{kl}	9.39 ±0.02 ^{k}	9.41 ±0.03 ^{k}	9.45 ±0.01 ^{j}
5	10.37 ±0.03 ^{i}	10.40 ±0.02 ^{hi}	10.41 ±0.02 ^{h}	10.43 ±0.03 ^{h}	10.47 ±0.02 ^{g}
10	11.39 ±0.03 ^{f}	11.42 ±0.02 ^{ef}	11.43 ±0.02 ^{de}	11.45 ±0.02 ^{cd}	11.47 ±0.01 ^{c}
15	13.43 ±0.02 ^{b}	13.45 ±0.02 ^{ab}	13.45 ±0.01 ^{ab}	13.47 ±0.01 ^{a}	13.48 ±0.01 ^{a}
Viability %	143.64	143.39	143.24	143.15	142.65

Table 5: Total viable counts of Bifidobacterium bifidum 2203 ATCC, of flavored fermented soymilk during storage period at 4 ± 1 °C for 15 days (log cfu/ml)

Storage period (day)	*Bifidobacterium bifidum 2203 ATCC* (log cfu/ml)				
	S1	S2	S3	S4	S5
Fresh	5.50 ± 0.20 ^{g}	9.37 ± 0.01 ^{d}	9.37 ±0.03 ^{d}	9.39 ±0.03 ^{d}	9.43 ±0.03 d
5	5.63 ± 0.05 ^{f}	10.39 ±0.01^{c}	10.39 ±0.02 ^{c}	10.41 ±0.03 ^{c}	10.45 ±0.03 c
10	5.75 ± 0.10 ^{e}	11.41 ±0.01 ^{b}	11.41 ±0.03 ^{b}	11.44 ±0.03 ^{b}	11.46 ±0.02 b
15	5.80 ± 0.07 ^{e}	13.43 ±0.02 ^{a}	13.44 ±0.03 ^{a}	13.47 ±0.02 ^{a}	13.48 ±0.01 a
Viability %	105.45	143.33	143.44	143.45	142.95

Table 6: Total viable counts of *Streptococcus thermophilus,* of flavored fermented soymilk during storage period at 4 ± 1 °C for 15 days (log cfu/ml)

Storage period (day)	*Streptococcus thermophilus* (log cfu/ml)				
	S1	S2	S3	S4	S5
Fresh	9.24 ±0.04 ^{l}	9.28 ±0.03 ^{k}	9.29 ±0.02 ^{k}	9.31 ±0.03 ^{k}	9.36 ±0.01 ^{j}
5	10.27 ±0.03 ^{i}	10.30 ±0.03 ^{hi}	10.31 ±0.02 ^{h}	10.34 ±0.03 ^{h}	10.38 ±0.01 ^{g}
10	11.30 ±0.03 ^{f}	11.33 ±0.02 ^{ef}	11.34 ±0.01 ^{e}	11.37 ±0.03 ^{e}	11.41 ±0.01 ^{d}
15	13.33 ±0.03 ^{c}	13.36 ±0.02 ^{bc}	13.38 ±0.02 ^{b}	13.40 ±0.02 ^{b}	13.45 ±0.01 ^{a}
Viability %	144.26	143.97	144.03	143.93	143.70

4. CONCLUSION:

This study's findings demonstrated that the incorporation of mango pulp into fermented soymilk, utilizing encapsulated yogurt culture and Bifidobacterium bifidum, elevated the concentrations of total phenolics, total flavonoids, and antioxidant activity while also enhancing the volatile components in the fermented soymilk. Additionally, using encapsulated bacteria enhanced the viability of bacteria in the product. Consequently, fermentation utilizing encapsulated yogurt culture and Bifidobacterium bifidum, along with the incorporation of mango pulp into fermented soy milk, can serve as a method to enhance fermented soymilk as a health food or health food ingredient with multifunctional attributes.

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الملخص العربي:

خصائص مضادات الأكسدة ومركبات النكهة المتطايرة في لبن فول الصويا المتخمر المنكه باستخدام بادئ الزبادي وبكتيريا البروبيوتك المغلفة

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تمت دراسة خصائص مضادات الأكسدة مكونات النكهة المتطايرة وحيوية البكتريا المغلفة في لبن فول الصويا المتخمر خلال فترة التخزين لمدة 15 يومًا. تم تحضير لبن فول الصويا المتخمر المنكه باستخدام لبن فول الصويا المتخمر باستخدام بادئ الزبادي وبكتريا *Bifidobacterium bifidum, 2203 ATCC* ثم اضافة لب المانجو له بنسب مختلفة (0 %، 10%، 20%، 30%). أدت إضافة لب المانجو الي لبن فول الصويا المتخمر باستخدام البكتريا المغلفة الي تحسين خصائص مضادات الأكسدة وزيادة مكونات الطعم والرائحة وزيادة حيوية البكتريا في المنتج اثناء التخزين. أوضحت النتائج ان اعلي زيادة كانت في المعاملة S5التي تحتوي على 30% لب مانجو مقارنة بالمعاملات (S1 , S2) التي لا تحتوي على لب المانجو.

أوضحت النتائج ان إضافة لب المانجو أدت الي زيادة كل من محتوي الفينولات الكلية من 66.41 (S1) الي 100.17 (S5)ملجم مكافئ حمض جاليك لكل مئة جرام عينة. وزيادة محتوي الفلافونيدات من 132.67 الي 166.65 ملجم مكافئ كيرسيتين لكل مئة جرام عينة. وزيادة نشاط مضادات الاكسدة من 69.35 % الي 76.34%. وزيادة تركيز الداي اسيتيل من 4.33 الي 6.13 جزء في المليون. وزيادة تركيز الأسيتيون من 8.64 الي 9.66 جزي في المليون بينما انخفض تركيز الأسيتالدهيد بإضافة لب المانجو من 2.64 و2.71 في المعاملات S1 , S2الي 2.27 جزء في المليون في المعاملة S5 المحتوية على 30% مانجو ثم يزاد تركيزه خلال التخزين الي 4.55 بمعدل اعلي من العينات الغير محتوية على المانجو. أيضا أدت إضافة المانجو الي زيادة حيوية البكتريا المغلفة في المنتج واستمرت هذه الزيادة خلال فترة التخزين وكانت اعلي زيادة في المعاملة المضاف اليها 30% لب مانجو.